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Immunodeficiency in Down's syndrome: T-lymphocyte subset imbalance in trisomic children

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SUMMARY

In fourteen children with Down's syndrome the percentage of circulating T-cells forming rosettes with sheep erythrocytes, either in the presence or in the absence of foetal calf serum, was significantly lower than in appropriately matched controls. In contrast the percentage of T-cells forming rosettes with human erythrocytes was significantly higher in children with Down's syndrome than in controls. These data support the hypothesis that a defective T-cell maturation is an early integral feature of Down's syndrome.

INTRODUCTION

It is well known that subjects with Down's Syndrome (DS) have an increased susceptibility to infections, a high risk of malignancies and increased frequency of autoantibodies (Öster, Mikkelsen & Nielsen, 1975; Miller, 1970; Fialkow, 1970; Burgio *et al.*, 1965). Fairly good experimental evidence has accumulated over the last few years suggesting that a combined deficiency of cell-mediated and antibody-mediated immunity might underlie these pathological phenomena (Whittingham *et al.*, 1977; Schlesinger *et al.*, 1976; Burgio *et al.*, 1975). As to cell-mediated immunity, it is well established that lymphocyte proliferative responsiveness to PHA in subjects with DS is normal during the first decade of life declining rapidly thereafter to pathologically low levels (Burgio *et al.*, 1975; Whittingham *et al.*, 1977; Seger, Buchinger & Str¨der, 1977). However, the percentage of circulating T-lymphocytes, assessed by the E-rosette method (formation of rosettes with SRBC) or by appropriate cytotoxicity testing, has been reported to be definitely low from birth onward (Levin, Nir & Mogilner, 1975; Ugazio *et al.*, 1977). The latter findings have suggested that a T-cell deficiency is a congenital (primary) feature of DS (Schlesinger *et al.*, 1976; Ugazio *et al.*, 1977).

However, in a recent comprehensive study of immunodeficiency in DS, Whittingham *et al.* (1977) reported a normal percentage of peripheral blood T-lymphocytes forming rosettes with 2-aminoethyl isothiuronium bromide-treated (AET) SRBC. These authors suggested that immunodeficiency in DS might be secondary to antigenic 'stress' due to rearing under unfavourable environmental conditions (institutionalization) and/or to inability to maintain adequate standards of personal hygiene.

The numerical estimates of circulating T-cells are known to vary according to the rosetting technique employed and this is probably related to a different expression of cell receptors by different T-cell subpopulations (Pellegrino *et al.*, 1975; Wybran, Carr & Fudenberg, 1972; Weiner, Bianco & Nussenzweig, 1973). Accordingly discrepancies in the literature concerning the percentage of circulating T-cells in DS could be the result of an imbalance in the proportions of different T-cell subsets.

To test this hypothesis three different assays for T-cells were carried out on the same blood sample in children with DS and in age-matched controls: formation of rosettes with SRBC in the presence of

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foetal calf serum (E_{FCS}-rosettes) and in the absence of FCS (E-rosettes), and formation of rosettes with human red blood cells (H-rosettes). The latter have been shown to be a subset of E-rosette-forming cells in normal subjects (Yu, 1975; Lanzavecchia *et al.*, 1977).

MATERIALS AND METHODS

Subjects. Fourteen children with DS ranging in age from 6 months to 4 years and fourteen karyotypically normal, mentally defective controls, matched for age and environmental conditions, were studied. All the children with DS were primary trisomics. The children of both groups were cared for at home and all were in good general health and taking no drugs at the time of the study.

E-rosettes and E_{FCS}-rosettes. Cells forming rosettes with SRBC were evaluated as described previously (Burgio *et al.*, 1975). Briefly, lymphocytes were separated from peripheral blood by centrifugation on Ficoll–Hypaque and 1×10^6 cells were mixed with 50×10^6 washed SRBC in a final volume of 1 ml of medium in U bottom plastic tubes. The medium was RPMI 1640 supplemented with 25% heat-inactivated FCS extensively absorbed with SRBC for E_{FCS}-rosettes, or RPMI 1640 alone for E-rosettes. The cell suspension was incubated at 37°C for 15 min, centrifuged at 200 *g* for 5 min and then incubated at 4°C for 1 hr. Cells were resuspended by rotating the tubes for 20 sec at 15 rev/min around a horizontal axis. The sample was aspirated into a pipette previously wetted with a solution containing 0·01 mg/ml acridine orange and applied to a hemocytometer chamber. Cells were examined simultaneously under UV and tungsten illumination and those binding three or more SRBC were considered rosettes. Under these conditions nuclei were stained bright green; polymorphs and monocytes occasionally contaminating the lymphocyte preparation were easily detected both by cellular morphology and by the presence of red lysosomes in the cytoplasm (Allison & Mallucci, 1964) and omitted in the count.

H-rosettes. Lymphocytes forming rosettes with human red blood cells (HRBC) were evaluated as described by Yu (1975) with slight modifications. Briefly, 1×10^6 lymphocytes and 12×10^6 washed group O HRBC were mixed in a 1·0 ml volume in U bottom plastic tubes. The medium was 199 Hanks supplemented with 5% BSA; after addition of BSA pH was titrated back to 7·0. Cells were incubated for 15 min at 37°C, centrifuged for 5 min at 200 *g* and left overnight at 4°C. The pellet was resuspended and examined as described for E-rosettes; cells binding two or more HRBC were considered H-rosettes.

Statistical evaluation. A Student's *t*-test was employed to compare the means. Since Student's *t*-test requires that the data be normally distributed and percentage data are usually not so distributed, all percentages were converted to degrees using the transformation $\mathrm{Sin}^{-1}\sqrt{P/100}$ (Fisher, 1957).

RESULTS

Irrespective of the presence of FCS in the incubation mixture, the percentage of E-rosettes was significantly lower in trisomics than in age-matched controls (Table 1). However, the mean increase in the percentage of rosettes induced by the addition of FCS was greater in DS (+19%) than in controls (+11%) and the difference was statistically significant ($P < 0{\cdot}02$).

The percentage of circulating H-rosettes was higher in subjects with DS than in age-matched controls (Table 1). H-rosettes constituted about a half of both E- and E_{FCS}-rosettes in normal subjects whereas their percentage was equal to or greater than that of both E- and E_{FCS}-rosettes in trisomic patients (Fig. 1).

TABLE 1. Results of three different T cell assays in children with Down's syndrome and in matched controls

	E_{FCS}%	E%	H%
DS ($n = 14$)	49·1±6·1 (39–53)	30·4±8·1 (23–39)	47·4±10·1 (27–69)
Controls ($n = 14$)	61·5±7·2 (53–76)	50·2±8·6 (38–64)	27·4±7·5 (10–39)
Significance (P value)	<0·001	<0·001	<0·001

Figures given are the average ± s. d. and range in parentheses. Student's *t*-test was performed after angular transformation of the data.

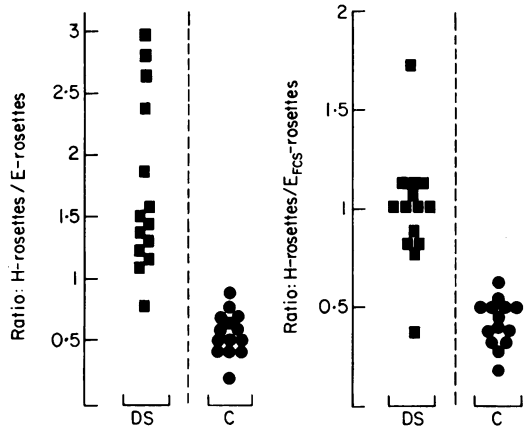

FIG. 1. Ratios of percentages of H-rosettes to percentages of E-rosettes with (E_{FCS}) or without (E) foetal calf serum in subjects with DS and in karyotypically normal controls.

DISCUSSION

The low percentages of peripheral blood lymphocytes forming rosettes with SRBC found in this study in children with DS confirm previous data from larger series (Burgio *et al.*, 1975; Ugazio *et al.*, 1977; Levin *et al.*, 1975) and reinforce the view that a selective T-cell deficiency is a congenital (primary) feature of DS.

The findings reported by Whittingham *et al.* (1977) of a normal percentage of E-rosettes in subjects with DS were obtained using AET-treated SRBC. Treatment of SRBC with AET has been shown to increase the avidity of binding to peripheral blood lymphocytes as well as the percentage of rosettes formed (Pellegrino *et al.*, 1975). In the present study the addition of serum to the incubation mixture, known to stabilize binding between erythrocytes and T-lymphocytes (Wybran *et al.*, 1972; Brain, Gordon & Willets, 1970), had a much less pronounced effect on the percentage of E-rosettes in normal subjects than in subjects with DS. The present findings, together with those of Whittingham *et al.* (1977), suggest that in DS most peripheral blood T-lymphocytes have a lower than normal avidity for SRBC.

It has been shown that H-rosettes are a subset of E-rosettes in the peripheral blood lymphocytes of normal subjects (Yu, 1975) and that this subset is characterized by a relatively low binding avidity for SRBC (Lanzavecchia *et al.*, 1977). The present finding of high percentages of H-rosettes in subjects with DS is therefore in keeping with the hypothesis that most circulating T-cells in DS belong to a subset of T-cells characterized by the capacity to bind HRBC and by low avidity for SRBC.

The immunological significance of this T-lymphocyte subset is not yet well understood. It has been claimed that H-rosetting is a property of relatively immature T-cells, so called T_1-cells (Bach & Carnaud, 1976), while further maturation to the more differentiated T_2-cells is accompanied by loss of this property. Differentiation from T_1 to T_2 cells is likely to be under the control of thymic hormonal factors (Bach & Carnaud, 1976). The data of Levin *et al.* (1975) as well as preliminary data from our own laboratory (Ugazio *et al.*, 1977) suggest that an early feature of the immunodeficiency in DS could be a decreased activity of thymic hormonal factors.

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